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Evaluation of *Verticillium lecanii* Strains Applied in Root Drenches for Suppression of *Meloidogyne incognita* on Tomato

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ABSTRACT: Three-week-old tomato seedlings were transplanted from sand into 10-cm-diameter pots (540-ml volume). Each pot contained 600 g loamy sand to which either 1,000 or 5,000 *Meloidogyne incognita* eggs were added. Five strains of the fungus *Verticillium lecanii* were individually applied in root drenches to the tomato plants at the time of transplanting, at an application rate of about 0.08% (dry weight fungus/dry weight loamy sand). The strains were a wild type strain and four mutants induced from that strain. Control plants were treated with water only or with autoclaved (nonviable) fungus. The experiments ended 45 days after transplanting, when the number of eggs per pot, root infection ratings, root lengths, and shoot dry weights were determined. The numbers of eggs counted from fungus-treated plants did not differ significantly from the numbers on water-only control plants. Application of autoclaved wild type strain to pots treated with 5,000 eggs resulted in an infection rating significantly higher than infection ratings recorded from several other fungus treatments and from plants treated with water only, but not in increased egg numbers.

KEY WORDS: Biological control, fungus, *Lycopersicon esculentum,* root-knot nematode.

Meloidogyne (root-knot nematode) is one of the most destructive genera of plant-parasitic nematodes, affecting numerous crop plants worldwide. The identification and successful deployment of biological control organisms would greatly benefit existing management programs for this plant pest. Numerous studies have focused on fungi as microbial pest control agents for the species *Meloidogyne incognita* (Kofoid & White) Chitwood on tomato (e.g., Jansson et al., 1985; Mankau and Wu, 1985; Gaspard, 1986; Ibrahim et al., 1987; Cabanillas et al., 1989; Gaspard et al., 1990a, b; Leij et al., 1992a, b, c; Santos et al., 1992; Duponnois et al., 1995; Gautam et al., 1995). A commercial product containing *Arthrobotrys* was developed for management of root-knot nematode on tomato plants, but is not in widespread use (Stirling, 1991). *Verticillium lecanii* (A. Zimmermann) Viégas is another fungus investigated as a potential management agent for *M. incognita* (Meyer, 1994). A mutant strain (selected for increased benomyl tolerance) and a wild type strain, both found to have action against *Heterodera glycines* (soybean cyst nematode) on soybean (Meyer and Meyer, 1995, 1996; Meyer and Huettel, 1996), were tested in the greenhouse for antagonism to *M. incognita* on tomato plants (Meyer, 1994). An alginate granule formulation was selected for the tomato study because it was efficacious in soybean tests with *V. lecanii* (Meyer and Huettel, 1993, 1996; Meyer and Meyer, 1995, 1996), and because alginate granules have been effectively employed in various tests of biocontrol fungi for plant-parasitic nematodes (e.g., Cabanillas et al., 1989; Schuster and Sikora, 1992a, b; Stirling and Mani, 1995). Application of the 2 *V. lecanii* strains in alginate granules decreased *M. incognita* populations on tomato in some experimental trials, but no application rate of either strain resulted in consistent, significant decreases in *M. incognita* populations (Meyer, 1994).

Because formulation is vital to success of biocontrol agents, the current study was initiated to determine whether a root drench would be efficacious for application of *V. lecanii* strains against root-knot nematode. The effects of root drenches applied at the time of tomato seedling transplant were investigated for the 2 previously tested *V. lecanii* strains and for the 3 other mutant strains that had been selected for benomyl tolerance (Meyer, 1992). All 5 strains were tested because biocontrol activity often varies with fungus strain.

Materials and Methods

Preparation of fungi and nematodes

Mutant strains M1S1, M2S1, M9S1, and M10S1 (Agricultural Research Service Culture Collection, NRRL #'s 18725, 18726, 18727, and 18728, respectively) were induced from a wild type strain of *Verticillium lecanii* (American Type Culture Collection 58909) with ultraviolet radiation and selected for increased tolerance to the fungicide benomyl (Meyer, 1992). For the greenhouse tests, the fungi were grown

for 3 days in 1-L erlenmeyer flasks (each flask containing 250 ml potato dextrose broth) that were rotated on orbital shakers (240 rpm) at 25°C (Meyer, 1994). Mycelium was harvested by centrifugation of the broth cultures at 13,000 *g* (Meyer, 1994). Conidia produced in the broth cultures were collected with the mycelium, but our studies on shelf life of these *V. lecanii* strains indicated that the conidia are short lived; and therefore, they are not considered useful for nematode management applications (unpubl). The collected mycelium was divided into 2 parts; half was autoclaved to be used as a nonviable control, while the other half was used live for addition to pots. Autoclaved treatments are given the suffix "A." All strains were refrigerated at 4°C overnight and used the day after harvest from the erlenmeyer flasks.

Meloidogyne incognita eggs for tomato plant infestation were collected from greenhouse cultures (Meyer, 1994).

Greenhouse experiments

Tomato (*Lycopersicon esculentum* Mill.) cv. Marglobe seeds were sown in sand in styrofoam flats. Seedlings (ca. 3 wk old) were transplanted into 540-ml pots (10 cm diameter), each containing 600 g (air-dried weight) of loamy sand. The loamy sand was made from a compost/sand mixture (3 parts compost to 1 part sand) with a final composition of 79% sand, 11% silt, 7% clay, 3% organic matter, pH 6.9. *Meloidogyne incognita* eggs were mixed into the loamy sand just prior to transplanting of the tomato seedlings. The root-knot nematode eggs were added at 2 rates: 1,000 and 5,000 eggs per pot. For transplanting, a depression large enough for the plant roots was made in the loamy sand of each pot. Fungus (live or dead) in 60 ml of water was added to the depression in each pot. The fungus application rate was ca. 0.5 g dry weight fungus per pot, equivalent to ca. 0.08% dry weight fungus per dry weight loamy sand. Controls without live or dead fungus received 60 ml of water only. A tomato seedling was immediately transplanted into each depression. Ten pots were used for each treatment, and the experiment was later repeated for a total of N = 20 pots per treatment (with a few exceptions where plants died during the course of the experiment). The pots were arranged in a randomized complete block design under daylight (greenhouse temperatures reaching up to ca. 40°C). Plants were fertilized with Sierra™ Poinsettia Mix at recommended rates. The experiments were terminated 45 days after transplanting, at which time the tomato plants were cut off just above the soil line, and shoot weights were determined after drying at 63°C.

Egg counts and infection class ratings

Root lengths were measured from the soil line to the tip of the main root. Nematode infection class numbers were assigned to roots (Daulton, 1959). Rating numbers indicate the following: 0 = free from galls; 1 = less than 5 galls; 2 = trace to 25 galls; 3 = 26 to 100 galls; 4 = moderate, numerous galls, mostly discrete; 5 = moderately heavy, numerous galls, many coalesced. Root-knot nematode eggs were collected as in Meyer (1994), except that egg masses from loamy sand that was very dry at harvest had to be collected on the 60-mesh sieve and broken apart in a mortar and pestle or a manual tissue grinder. The eggs were then collected on a 500-mesh screen (pore size 25 μm) and counted.

Isolation of fungi from loamy sand

To test for the presence of the fungus at the end of the experiment, loamy sand from each pot was stirred in water (0.02% dry weight per volume water), plated onto semiselective media (0.05 ml of suspension per petri dish), and incubated at 25°C. Two media were used. One medium was PDA ABE 1000, similar to PDA ABE 100 (Meyer, 1994). Each liter of PDA ABE 1000 contained 39 g of PDA (potato dextrose agar), 970 ml of distilled water, 2 gr of benlate in 20 ml of distilled water (Benlate 50 Wettable Powder or DF, E. I. Dupont de Nemours & Co., Wilmington, Delaware), and antibiotics (0.3 g of streptomycin sulfate plus 0.3 g of tetracycline in 10 ml of sterile water). Six milliliters of EtOH were used to rinse the flask in which the antibiotics were mixed and were then added to the medium. The second medium was modified Ausher's Medium Number 2 (Ausher et al., 1975), with PCNB replaced by benomyl. Loamy sand suspensions were plated out as follows: (1) water controls: 1 petri dish of Ausher's medium and 1 petri dish of PDA ABE 1000 per pot; (2) wild type strain treatments: 2 petri dishes of Ausher's medium per pot; and (3) mutant strain treatments: 2 petri dishes of PDA ABE 1000 per pot. Consequently, loamy sand from water control treatments was plated onto 78 petri dishes (1 pot was not sampled), and loamy sand from each fungus treatment was plated onto 80 petri dishes.

Analysis of data

The experiments were combined and analyzed as an incomplete block design using the SAS procedure MIXED (SAS, 1992). The egg count values were wide ranging, differing in magnitude by as much as a factor of 10; the variances of the treatments were heterogeneous. To correct this, the data were $\log_{10}$ transformed. The variables: $\log_{10}$ (egg count), infection class, root length, and shoot dry weight were analyzed as mixed models. Experiment and the experiment by treatment interaction were considered random effects.

Results and Discussion

When 1,000 eggs were initially applied to pots, the largest reductions in egg populations resulted from individual application of M1S1A, live wild type strain, and wild type A (Table 1). The latter 2 treatments resulted in a 32% reduction in egg numbers compared to water controls (based on egg count means transformed from $\log_{10}$ egg counts), and M1S1A treatment resulted in a 28% decrease. However, the reductions were not significantly different from the egg numbers found in pots treated with water only. In the pots that had received 1,000 eggs, 2 of the 3 lowest infection ratings were recorded

Table 1. Effects of water, live *Verticillium lecanii,* and autoclaved *Verticillium lecanii* on tomato plants, number of galls, and mean numbers of *Meloidogyne incognita* eggs produced per pot. Pots initially received either 1,000 or 5,000 eggs.

	Number of eggs*		Infection rating		Shoot dry weight—grams†	
Treatment	1,000	5,000	1,000	5,000	1,000	5,000
Water	1,920	6,272 ab§	2.4	3.2 ab	38.7 a	40.0 bc
M1S1A‡	1,390	9,282 b	2.7	3.8 bc	41.4 c	39.5 abc
M1S1	2,124	4,982 ab	2.6	3.6 abc	40.0 abc	40.6 bc
M2S1A	1,745	3,011 a	2.4	3.0 ab	38.0 a	38.2 ab
M2S1	1,500	7,037 b	2.2	3.1 abc	40.1 bc	37.8 ab
M9S1A	2,408	4,226 ab	2.7	3.3 abc	38.7 ab	37.1 ab
M9S1	1,633	5,349 ab	2.6	3.1 abc	40.9 bc	40.3 bc
M10S1A	1,882	4,597 ab	2.0	2.9 ab	41.0 bc	39.4 ab
M10S1	2,600	5,083 ab	2.2	2.9 a	39.8 abc	41.5 c
Wild type A	1,309	5,780 ab	1.9	3.8 c	39.0 abc	37.7 ab
Wild type	1,308	7,254 ab	2.1	3.5 abc	38.7 ab	38.3 ab
F-value	6.39		5.59		2.52	
P-value	0.0001		0.0001		0.0108	

* Values are the egg count means back transformed from $\log_{10}$.

† Values are least squares means.

‡ Treatments with letter A represent autoclaved fungal strains.

§ Numbers followed by the same letter are not significantly different at $P = 0.05$, based on analysis of $\log_{10}$-transformed data. Letters are comparable within columns but not between columns. Any apparent discrepancies in letter assignments (such as 2.9 a and 2.9 ab in the 5,000 infection rating column) are due to the fact that the standard error of the difference between the means is not the same for all pairs.

from the 2 wild type strain treatments (Table 1), but the infection ratings were similar for all treatments, generally falling in the 2–3 range (from more than 5 galls to less than 100). Root lengths among treatments were similar ($P > 0.05$), ranging from 12.1 cm to 16.8 cm (least squares mean per treatment). The highest shoot dry weights were in plants treated with M1S1A (Table 1).

Trends recorded at 1 nematode population density did not appear at the other. When 5,000 eggs were initially added to each pot, the largest reduction in egg numbers (52% compared with water-treated controls) was recorded after treatment with M2S1A (Table 1). Despite the overall large reduction, there was high variability, and the 2 values were not significantly different. However, in the pots receiving 5,000 eggs, treatment with M2S1A did result in significantly fewer eggs than treatment with viable strain M2S1 or with M1S1A, and in one of the smallest root infection ratings. Applications of M1S1A or of the live wild type strain, which were associated with low egg counts when 1,000 nematodes were added per pot, were connected with high egg populations in the pots receiving 5,000 eggs. Additionally, M1S1A and wild type A treatments resulted in significantly higher infection class ratings than some other treatments in the pots that received 5,000 eggs. Most of the other infection class ratings were similar to each other (ca. 3–4; from 26 galls to more than 100, galls mostly discrete), although plants in pots treated with strain M10S1 and 5,000 eggs had low infection ratings and also had the heaviest shoots (Table 1).

Delivery of a potential biocontrol fungus during transplant of tomato seedlings was previously tested with *Monacrosporium ellipsosporum* (Grove) Subr., which was applied on wheat grain substrate to tomato seedling transplant holes (Mankau and Wu, 1985). That study demonstrated a trend toward nematode suppression in fungus-treated plants, but there was not a significant difference from controls. Similarly, in the current study, none of the 5 strains applied to transplant holes significantly affected root-knot nematode populations on tomato plants. The lack of food source for the fungus in the root drench may have outweighed any advantage of direct root contact from the drench application, although *V. chlamydosporium* and *Monacrosporium ellipsosporum* were effective against *Meloidogyne* spp. on tomato when applied to soil without a food source (Leij and Kerry, 1991; Santos et al., 1992). Indeed, *V. chlamydospor-*

ium established in soil more readily without a food base, presumably because other microorganisms could feed upon the bran (Leij and Kerry, 1991). In the current experiment, there were only 4 isolations of *V. lecanii* from greenhouse pots: strain M2S1 was isolated from 1 pot (5,000-egg treatment) and strain M9S1 from 3 pots (2 pots treated with 5,000 eggs and 1 pot with 1,000 eggs). The 4 mutant strains of *V. lecanii* reduced *Heterodera glycines* populations even with poor fungus isolation rates from greenhouse pots (Meyer and Meyer, 1995, 1996; Meyer and Huettel, 1996). Consequently, it is not known whether *V. lecanii* was ineffective in the current experiment because of failure to survive in the soil, or because of a low level of activity against *M. incognita.* In either case, the strains were not efficacious management agents for this nematode.

Treatment with autoclaved alginate granules containing nonviable fungus did not affect *M. incognita* populations (Meyer, 1994), so no effects were anticipated following treatment with autoclaved fungus in root drenches. Interestingly, in the root drench application, treatment with 1 autoclaved fungus strain resulted in lower egg numbers than treatment with viable *V. lecanii* of the same strain. Application of M2S1A to pots treated with 5,000 eggs resulted in significantly fewer eggs (57% reduction in egg numbers) than were produced in pots treated with live M2S1. This may have been due to such factors as antagonistic breakdown products from decomposing mycelium or to increased populations of microorganisms feeding on the dead fungus, but these parameters were not measured in this study. In the pots receiving 5,000 eggs, M2S1A was also more effective at reducing egg populations than M1S1A (68% difference in final egg counts), and resulted in lower root infection ratings than application of wild type A. This suggests that there is some difference among the strains, although there are insufficient data at this time to determine what that difference might be. Leij et al. (1992c) found that as *M. incognita* densities increased, live *Verticillium chlamydosporium* became less effective against the nematode. In the current experiment, activity of M2S1A appeared to be enhanced by increasing the nematode population density, since none of the differences in egg numbers or infection ratings were found in pots treated with 1,000 eggs. If autoclaved strain M2S1 had demonstrated remarkable suppression of *M. incognita,* it might be worthwhile to investigate impact on juveniles as well as on eggs, and to pursue application as an amendment or to study breakdown products from the dead mycelium. However, the effect compared with the water controls was not large enough to warrant study as a potential management agent.

A fungus that is effective against 1 plant-parasitic nematode is not necessarily active against another species. These strains of *Verticillium lecanii,* while efficacious against *H. glycines* under various greenhouse conditions (Meyer and Meyer, 1995, 1996; Meyer and Huettel, 1996), did not demonstrate similar activity against *M. incognita.*

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